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Two new species of *Strigula* (lichenised Dothideomycetes, Ascomycota) from China, with a key to the Chinese foliicolous species

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Abstract

Strigula has traditionally been circumscribed based on morphology, but species delimitation in the genus generally lacks comprehensive analyses. A molecular approach has now been applied to foliicolous material of the genus from tropical areas in China. On the basis of combined phenotyic and genotypic data, two new species are described from southern China: *S. acuticonidiarum* and *S. guangxiensis*.

Key words

Foliicolous lichens, lichens, molecular phylogeny, *Strigulales*

Introduction

Strigula Fr. is a genus of lichenised fungi belonging to the family Strigulaceae in the order Strigulales in the class Dothideomycetes (Hyde et al. 2013, Nelsen et al. 2009) of the phylum Ascomycota. It was the first lichenised genus ever noticed on tropical leaves by Elias Fries in 1821 and named in 1823 (Santesson 1952, Fries 1823: 535). About 70 species are known (Lücking 2008, Hyde et al. 2013), of which 21 have been reported from China. These comprise 15 foliicolous species: *Strigula antillarum* (Fée) R. Sant. (Jiang et al. 2016), *S. concreta* (Fée) R. Sant. (Aptroot 2003, Aptroot et al. 2003), *S. macrocarpa* Vain. (Wei 1991, Aptroot et al. 2003), *S. maculata* (Cooke & Massee) R. Sant. (Aptroot et al. 2003), *S. melanobapha* (Kremp.) R. Sant. (Santesson

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1952, Wei 1991, Lücking 2008), *S. minor* (Vezda) Sérus. (Aptroot et al. 2003), *S. nemathora* Mont. (Aptroot et al. 2003), *S. nitidula* Mont. (Aptroot et al. 2003), *S. phyllogena* (Müll. Arg.) R.C. Harris (Aptroot et al. 2003), *S. prasina* Müll. Arg. (Jiang et al. 2016), *S. schizospora* R. Sant. (Aptroot and Seaward 1999), *S. sinoaustralis* S.H. Jiang, X.L. Wei & J.C. Wei (Jiang et al. 2016), *S. smaragdula* Fr. (Santesson 1952, Wei 1991, Aptroot 2003, Aptroot et al. 2003), *S. subelegans* Vain. (Wei 1991, Wei and Jiang 1991), and *S. subtilissima* (Fée) Müll. Arg. (Santesson 1952, Aptroot and Seaward 1999, Aptroot et al. 2003), and six corticolous or saxicolous ones: *Strigula jamesii* (Swinscow) R.C. Harris (Aptroot 2003), *S. laureriformis* Aptroot & Lücking (Jiang et al. 2016), *S. muriformis* Aptroot & Diederich (Aptroot 2003), *S. phaea* (Ach.) R.C. Harris (Aptroot and Sipman 2001), *S. submuriformis* (R.C. Harris) R.C. Harris (Aptroot and Seaward 1999), and *S. viridiseda* (Nyl.) R.C. Harris (Aptroot 2003).

During our studies of the lichens of China, two species of *Strigula* new to science have been found as a result of integrated phenotypic and molecular analyses.

Materials and methods

Phenotypic analyses

All specimens of *Strigula* examined in this study were collected by the first author from Guangxi Province in China and are preserved in the Fungarium of the Institute of Microbiology, Chinese Academy of Sciences (HMAS–L). A Leica M125 dissecting microscope was used for the morphological studies and a Zeiss Axioscope2 compound microscope with a Zeiss Axio Imager A2 was used for the anatomical studies. Sections were studied and photographed with an AxioCam MRc5 camera in tap water. Thin-layer chromatography (TLC) (Culberson and Kristinsson 1970, Culberson 1972, Orange et al. 2001) was applied for the detection of lichen substances.

Genotypic analyses

Eighteen fresh specimens of *Strigula* were chosen for DNA extraction (Table 1), for which a modified CTAB method (Rogers and Bendich 1988) was used. Primers ITS5 and ITS4 were used to amplify the nrRNA gene ITS region (White et al. 1990). Reactions were carried out in a 25 µl reaction volume including 1 µl DNA, 1 µl each primer (10 µM), 2 µl dNTP (2.5 mM), 2.5 µl amplification buffer (containing 25 mM Mg^{2+}), 0.5 µl Taq polymerase and 17 µl ddH_2O. Cycling parameters were set to an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 50 s and a final extension at 72°C for 10 min. The new sequences generated for this study are deposited in GenBank (Table 1).

Twenty-seven sequences were aligned with the program MAFFT (Katoh 2002), including 18 newly generated for this study (Table 1). Seven sequences were taken

Table 1. Specimens of *Strigula* spp. from China and outgroup species used in the phylogenetic analyses.

Species	Fungarium No.	GenBank Accession No.
S. acuticonidiarum	HMAS–L 0138045	**KY100290**†
S. acuticonidiarum	HMAS–L 0138048	**KY100291**†
S. acuticonidiarum	HMAS–L 0138049	**KY100292**†
S. acuticonidiarum	HMAS–L 0138050	**KY100293**†
S. acuticonidiarum	HMAS–L 0138051	**KY100294**†
S. acuticonidiarum	HMAS–L 0138046	**KY100295**†
S. acuticonidiarum	HMAS–L 0138047	**KY100296**†
S. antillarum	HMAS–L 0137209	KX216696
S. antillarum	HMAS–L 0137208	KX216697
S. antillarum	HMAS–L 0137211	KX216702
S. antillarum	HMAS–L 0130571	**KY100288**†
S. antillarum	HMAS–L 0130573	**KY100289**†
S. guangxiensis	HMAS–L 0138040	**KY100301**†
S. guangxiensis	HMAS–L 0138065	**KY100303**†
S. guangxiensis	HMAS–L 0138044	**KY100302**†
S. guangxiensis	HMAS–L 0138041	**KY100304**†
S. guangxiensis	HMAS–L 0138042	**KY100305**†
S. prasina	HMAS–L 0137213	KX216700
S. prasina	HMAS–L 0137212	KX216701
S. sinoaustralis	HMAS–L 0137203	KX216699
S. sinoaustralis	HMAS–L 0137204	KX216698
S. smaragdula	HMAS–L 0138066	**KY100296**†
S. smaragdula	HMAS–L 0130621	**KY100298**†
S. smaragdula	HMAS–L 0138068	**KY100299**†
S. smaragdula	HMAS–L 0138067	**KY100300**†
Falciformispora senegalensis	IP614.60	KP132365
F. tompkinsii	IP559.60	KP132366

† The GenBank numbers in bold type were newly generated in this study.

from Jiang et al. (2016). Due to a lack of ITS sequences from other genera of *Strigulales*, *Falciformispora tompkinsii* (El-Ani) S.A. Ahmed et al. and *F. senegalensis* (Segretain et al.) S.A. Ahmed et al. from *Pleosporales* in the same class (*Dothideomycetes*) were chosen as outgroup (Jiang et al. 2016). The alignment was subjected to a Randomized Axelerated Maximum Likelihood (RAxML) analyses (Stamatakis et al. 2005, Stamatakis 2006), with parametric bootstrapping using 1000 replicates under the GTRGAMMA model chosen by running JModeltest (Posada 2008). Alignments are deposited in TreeBASE (http://www.treebase.org/) under accession number 20186.

Results and discussion

The Maximum Likelihood tree, based on the 27 ITS sequences (472 bp), is shown in Figure 1. Within the phylogenetic tree, two new species being named here, *Strigula*

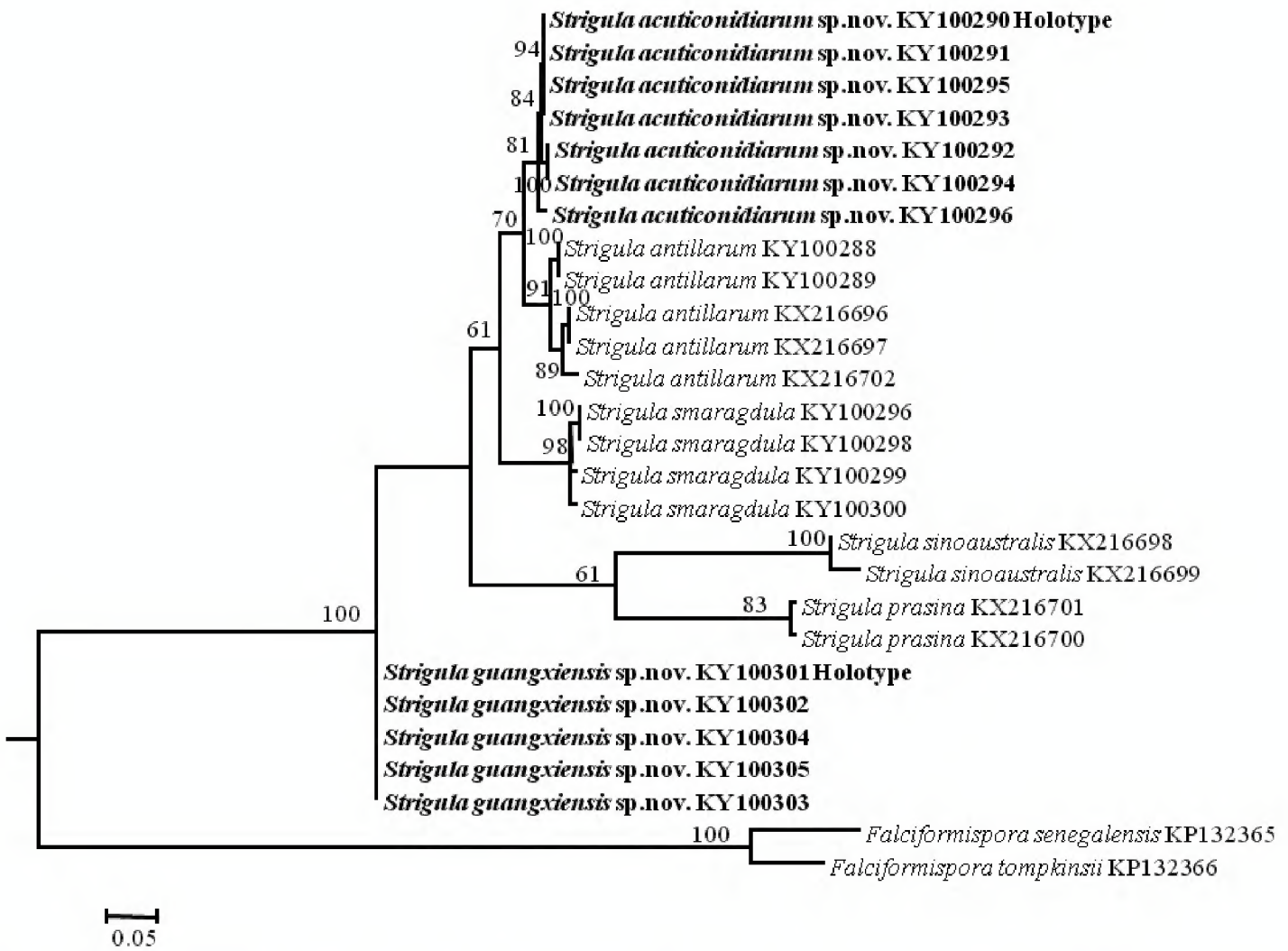

Figure 1. The Maximum Likelihood tree based on nrDNA ITS sequences with 472 bp. Genetic distance scale = 0.05 changes per site. Numbers above each node represents bootstrap support values (value lower than 50 not shown). New species proposed are in boldface.

acuticonidiarum S.H. Jiang, X.L. Wei & J.C. Wei and *S, guangxiensis* S.H. Jiang, X.L. Wei & J.C. Wei formed independent monophyletic groups and intraspecies variation between individuals within *S. acuticonidiarum* was also evident. *Strigula antillarum* appeared as a sister species to *S. acuticonidiarum*. In addition, *S. smaragdula*, *S. sinoaustralis*, and *S. prasina* formed separate branches.

Taxonomy

Strigula acuticonidiarum **S.H.Jiang, X.L.Wei & J.C.Wei, sp. nov.**
Fungal Names: FN570329
Figure 2a–d

Diagnosis. Differs from *Strigula antillarum* in the almost entirely immersed perithecia, and longer macroconidia with more acute ends.

Type. CHINA. Guangxi: Nanning City, Long'an County, Longhu mountain natural reserve. 22°57'42"N, 107°37'40"E, 150 m alt., on living leaves, 1 Dec 2015, S.H.Jiang GX201511085 (HMAS–L 0138045 – holotype).

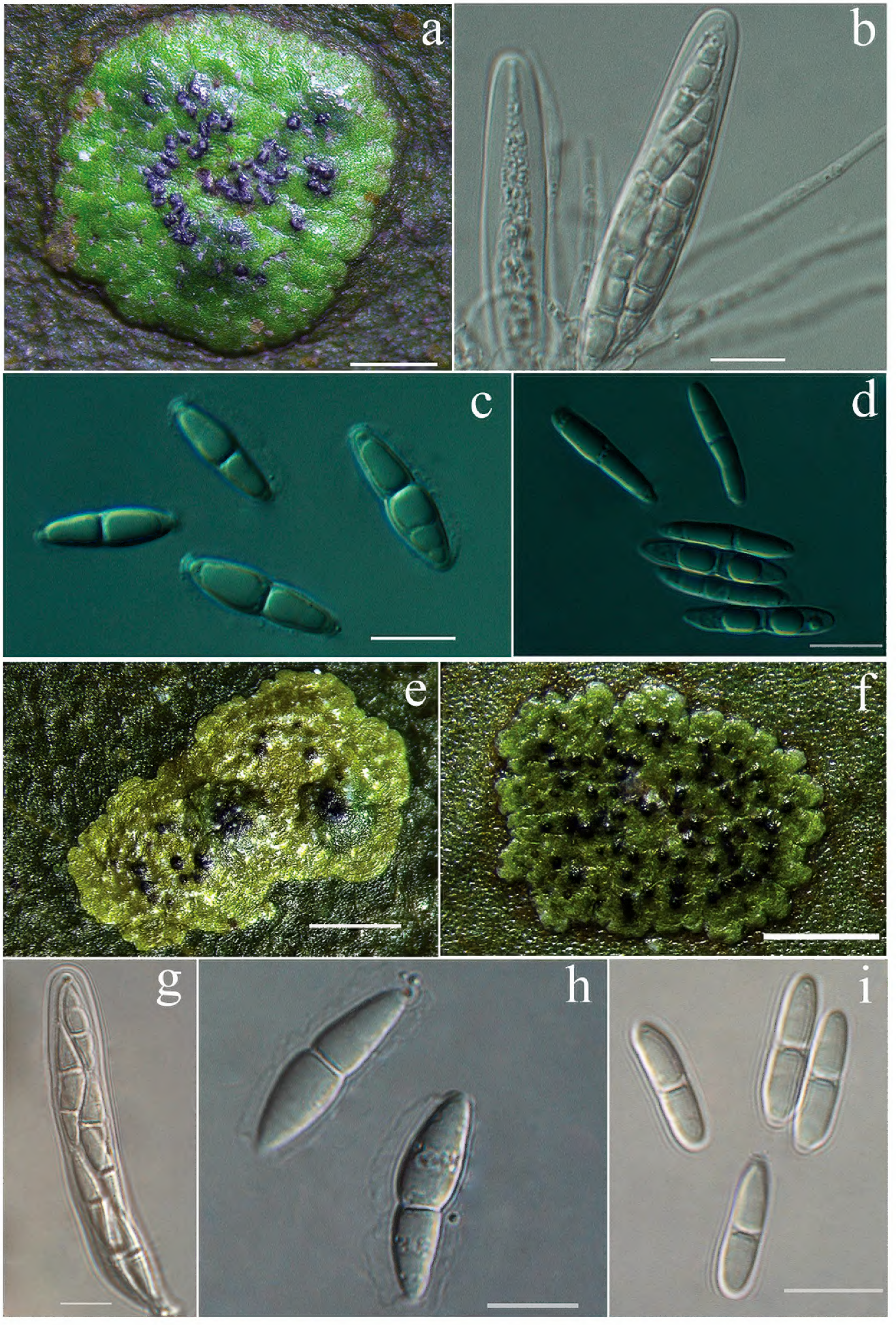

Figure 2. The new species *Strigula acuticonidiarum* (holotype, HMAS–L 0138045). **a** Thallus with perithecia and pycnidia **b** Asci, with eight biseriate ascospores **c** Ascospores, with constriction at septum **d** Macroconidia. *Strigula smaragdula* **e** Thallus with perithecia (HMAS–L 06211) **f** Thallus with pycnidia (HMAS–L 0138067) **g** Ascus, with eight biseriate ascospores (HMAS–L 0138066) **h** Ascospores, with 1-septate (HMAS–L 0138066) **i** Macroconidia (HMAS–L 0138066). Scale bars: **a, e, f** = 300 μm; **b, d, h** = 10 μm.

Description. Thallus subcuticular, continuous or dispersed into rounded to partly confluent patches, 0.5-4 mm across and 10-25 µm thick, margin entire to crenulate, distinct lobes absent, bright to dark green. Photobiont *Cephaleuros*, cells angular-rounded, 5–15 × 4–10 µm. Perithecia immersed to erumpent, covered by thalline tissue, mostly up to the ostiole, hemispherical, 0.3–0.5 mm diam and 100–140 µm tall, dark green, but the uppermost part often black. Exciple prosoplectenchymatous, 10–25 µm thick, colourless to brown. Involucrellum carbonaceous, 22.5–55 µm thick, black. Interascal filaments unbranched, *c.* 1–2 µm thick. Asci obclavate, 50–65 × 8–12 µm. Ascospores 8 per ascus, biseriate, fusiform, 1-septate, distinctly constricted at the septum, 12.5–20 × 3.7–5 µm, 2.5–5.5 times as long as broad. Pycnidia producing macroconidia numerous, black, wart-shaped, 0.1–0.15 mm diam. Macroconidia 1-septate, often constricted at the septum, ends acute, usually with 1–2 oil droplets per cell when fresh, 15–22.5 × 3–4 µm, 4–7.5 times as long as broad. Microconidia not seen.

Chemistry. No substances detected by TLC.

Habitat and distribution. On the surface of living leaves in humid, semi-exposed forests of south China.

Etymology. The epithet "*acuticonidiarum*" is a compound of a Latin adjective "*acutatus*" (*a, um,* and *acuti-* in Latin comp.) meaning sharply pointed, and "*macroconidiarum*", a plural genitive of the Latin neuter noun, "*macroconidium*". This recalls the acute ends of the macroconidia.

Other specimens examined. CHINA. Guangxi: Nanning City, Long'an County, Longhu mountain natural reserve. 22°57'42"N, 107°37'40"E, 150 m alt., on living leaves, 1 Dec 2015, S.H.Jiang GX201511068 (HMAS–L 0138049), GX201511069 (HMAS–L 0138048), GX201511070 (HMAS–L 0138046), GX201511080 (HMAS–L 0138053), GX201511084 (HMAS–L 0138050), GX201511089 (HMAS–L 0138051), GX201511094 (HMAS–L 0138052). Yunnan: Xishuangbanna, Mengla County, tropical botanical garden of Chinese Academy of Sciences, East area. 21°55'39"N, 101°15'52"E, 560 m alt., on living leaves, 18 Nov 2015, X.L.Wei & S.H.Jiang XTBG2015038 (HMAS–L 0138047).

Remarks. *Strigula antillarum* can be distinguished from the new species by the perithecia being immersed only at base, aggregate and confluent pycnidia forming black spots or radiating lines, and bacillar, shorter, macroconidia (12-20 × 3-4 µm) with rounded ends (Lücking 2008). The new species is externally most similar to *S. smaragdula* (Figure 2e–i), in which perithecia are covered by the bright green thallus (Santesson 1952, Lücking 2008). However, *S. acuticonidiarum* is characterized by a thinner thallus with entire to crenulate margins (thallus 20-80 µm thick in *S. smaragdula*), the absence of distinct lobes, and in having small and round thalli instead. Anatomically, it differs in the shorter asci and the macroconidia having more acute ends. In molecular analyses, the ITS rDNA sequences confirmed it as different from *S. smaragdula* (Figure 1). The two species are distinct both morphologically and phylogenetically.

Strigula smaragdula is generally considered to be a very common but variable species, traditionally recognized morphologically, for example by the thallus having entire to crenulate or lobulate margins, and sometimes the whole thallus being lobate-lacin-

iate. This variation series has been regarded as merely due to environmental or habitat modification. However, the most common state, represented by the holotype of *S. smaragdula*, is characterised by distinct, but short and rounded marginal lobes (Santesson 1952). The broad concept of *S. smaragdula* evidently represents a species complex, rather than a single species. Minor morphological traits, including thallus form and differences in ascus size and the shape of macroconidia, are diagnostic for segregating *S. acuticonidiarum* from *S. smaragdula s. str.*, a distinction supported by molecular data (Figure 1).

***Strigula guangxiensis* S.H.Jiang, X.L.Wei & J.C.Wei, sp. nov.**
Fungal Names: FN570330
Figure 3

Diagnosis. Characterized by the thin thallus (30–45 µm thick), long asci (45–65 × 10–12.5 µm), aggregated pycnidia, large ascospores (15–25 × 2.5–5 µm), and 1-septate macroconidia (12.5–17.5 × 2.5–5 µm).

Type. CHINA. Guangxi: Nanning, Long'an County, Longhu mountain natural reserve. 22°57'42"N, 107°37'40"E, 150 m alt., on living leaves, 1 Dec 2015, S.H.Jiang GX201511127 (HMAS–L 0138040 – holotype).

Description. Thallus subcuticular, dispersed into rounded to irregular, partly confluent patches, 1–2 mm across, a few to 3 mm, 30–45 µm thick, margins entire to crenulate, bright green to pale green, sometimes white in the centre, surface smooth. Photobiont *Cephaleuros*, cells 5–12 × 4–9 µm. Perithecia hemispherical, rarely found in specimens with aggregated pycnidia, small, scattered, round individuals with one or two perithecia occur in pure populations, basal part immersed in the thallus, 0.5–0.7 mm diam and 90–120 µm tall, black. Exciple prosoplectenchymatous, 7.5–12.5 µm thick, brown. Involucrellum carbonaceous, black, 20–90 µm thick. Interascal filaments unbranched, *c.* 1–2 µm thick. Asci obclavate, 45–65 × 10–12.5 µm. Ascospores 8 per ascus, biseriate, fusiform, 1-septate, distinctly constricted at the septum, distal cell slightly enlarged, 15–25 × 2.5–5 µm, 4–5 times as long as broad. Pycnidia producing abundant macroconidia, few on thalli producing perithecia and overgrowing them, single or most frequently aggregated in groups of 3–10, semi-immersed, wart-shaped, those producing macroconidia 0.07–0.15 mm diam, those producing microconidia 0.05–0.1 mm diam, black. Macroconidia bacillar, 1-septate, 12.5–17.5 × 2.5–5 µm. Microconidia fusiform, non-septate, 4–5 × 1.5–2 µm.

Chemistry. No substances detected by TLC.

Habitat and distribution. On the surface of living leaves in humid, semi-exposed forests of south China.

Etymology. The epithet "*guangxiensis*" is the name of the province including the type locality of the new species.

Other specimens examined. CHINA. Guangxi: Nanning, Long'an County, Longhu mountain natural reserve. 22°57'42"N, 107°37'40"E, 150 m alt., on living

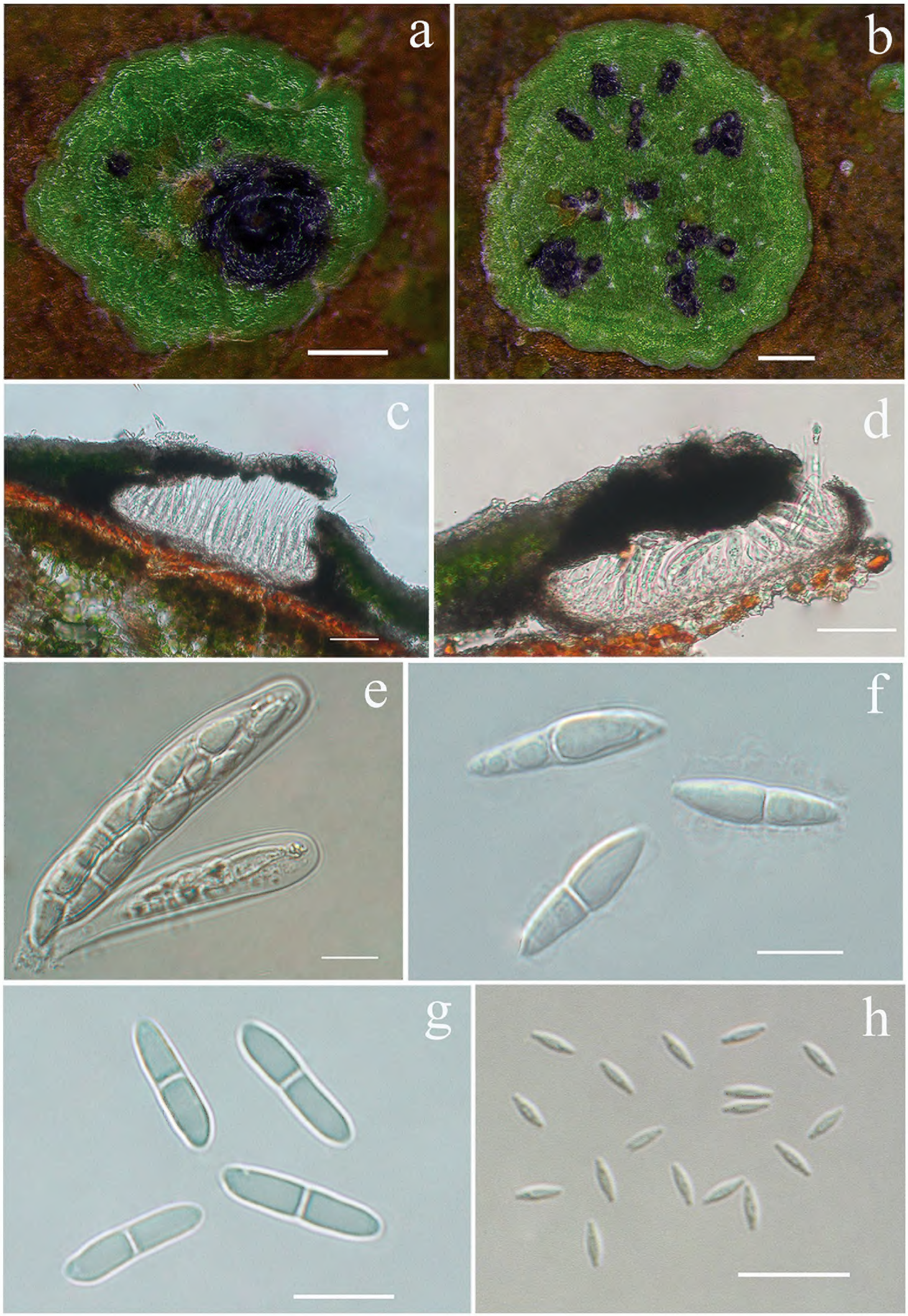

Figure 3. The new species *Strigula guangxiensis* (holotype, HMAS–L 0138040). **a** Thallus with perithecia **b** Thallus with pycnidia **c, d** Perithecia **e** Asci with eight biseriate ascospores **f** Ascospores, with distal cell slightly enlarged **g** Macroconidia; h Microconidia. Scale bars: **a, b** =100 μm; **c, d** =50 μm; **e, f, g, h** =10 μm.

leaves, 1 Dec 2015, S.H.Jiang GX201511078 (HMAS–L 0138044), GX201511087 (HMAS–L 0138041), GX201511071 (HMAS–L 0138065), GX201511107 (HMAS–L 0138043), GX201511130 (HMAS–L 0138042).

Remarks. *Strigula guangxiensis* is most similar to *S. subelegans*, having essentially the same ascospore dimensions, but differs in the smaller and thinner thallus (5–15 mm across and 30–70 µm thick in *S. subelegans*; Lücking 2008). In addition, the perithecia and pycnidia are usually separated on different thallus patches and the pycnidia are often aggregated (dispersed in *S. subelegans*) (Lücking 2008). *Strigula wandae* M. Cáceres & Lücking is also similar in appearance, but distinguished by the oblong-ellipsoid ascospores, with cells of equal size, solitary pycnidia, and smaller macroconidia (12–15 × 2.5–3 µm) (Lücking et al. 2003, Lücking 2008).

With respect to aggregated pycnidia, four other species of *Strigula* have aggregated pycnidia developing as in similarly with *S. guangxiensis*: *S. schizospora*, which can be distinguished by the smaller ascospores (8–12 × 2–2.5 µm), usually breaking into parts while still within the asci, and the smaller macroconidia (4–6 × 1.5–2 µm) (Santesson 1952); *S. lacericola* P.M. McCarthy, has smaller, narrow ascospores (10–14 × 1.5–2.5 µm), with cells of equal size and smaller, and non-septate macroconidia (6–8 × 1–2 µm) (McCarthy 2009); *S. novae-zelandiae* (Nag Raj) Sérus., characterised by the circular thalli with a crenulate to deeply digitate margin and especially the pycnidia producing polarilocular macroconidia (Sérusiaux 1998); and *S. antillarum*, which has a thinner thallus (20–30 µm thick) and longer asci (60–70 × 8–11 µm) (Lücking 2008). According to our phylogenetic analyses, even though the differences in morphology are subtle, the species are readily separated in the molecular phylograms (Figure 1).

Key to the foliicolous *Strigula* species reported from China

1. Thallus usually hypophyllous, usually on the lower leaf surface; interascal filaments richly branched and anastomosing....**1. *S.prasina*** (Hainan Province; Jiang et al. 2016)
1'. Thallus usually epiphyllous, usually on the upper leaf surface; interascal filaments simple or sparingly branched, rarely anastomosing
  2. Thallus supracuticular, easily separated from the leaf; alga *Phycopeltis*
    3. Perithecia greyish black to black (naked or covered by a thin, thallus layer), sharply delimited from the pale grey thallus, lens-shaped to applanately conical.....**2. *S. phyllogena*** (Yunnan Province; Aptroot et al. 2003)
    3'. Perithecia greyish green (covered with thalline tissue), not sharply delimited from the thallus, hemispherical to wart-shaped or conical.....**3. *S.minor*** (Yunnan Province; Aptroot et al. 2003)
  2'. Thallus subcuticular, not separable from leaf; alga *Cephaleuros*
    4. Involucrellum colorless, only in upper parts dark.....**4. *S. nemathora*** (Taiwan, Yunnan Province; Aptroot et al. 2003)
    4'. Involucrellum black
      5. Ascospores breaking into halves while still within the asci, asci appearing with to 16 simple ascospores

6. Thallus rather thick (30–50 μm), bright green to yellowish green, but often white in the centre. Perithecia half-immersed, only their black tops exposed.....**5. *S. schizospora*** (Hongkong; Aptroot and Seaward 1999)

6'. Thallus thin (10–30 μm); perithecia fully exposed or only basally immersed; pycnidia evenly dispersed over the thallus

7. Thallus very thin (10–15 μm), dark metallic green, often bordered by an irregular, thin, black line .....**6. *S. nitidula*** (Yunnan Province; Aptroot et al. 2003)

7'. Thallus thicker (15–35μm), not bordered by a thin, black line

8. Thallus pale greenish to bluish grey; asci 30–70 × 4–6 μm.....**7. *S. concreta*** (Yunnan Province; Aptroot 2003, Aptroot et al. 2003)

8'. Thallus with white-punctate; Asci 72.5–92.5 × 4–5 μm .....**8. *S. sinoaustralis*** (Guangdong Province, Guangxi Province; Jiang et al. 2016)

5'. Ascospores not breaking into halves while within the asci, but sometimes outside asci in squash mounts

9. Thallus very thin (8–15μm), metallic green to dark green or greenish brown and usually bordered by thin, black, sometimes interrupted line

10. Ascospores fusiform, 14–23 × 3–5 μm; macroconidia 10–12 μm long; black line interrupted.....**9. *S.melanobapha*** (Fujian Province, Yunnan Province; Santesson 1952, Wei 1991, Lücking 2008)

10'. Ascospores oblong-bacillar, 8–18 × 2–3 μm; macroconidia 4–7 μm long; black line continuous

11. Ascospores 8–12 μm long, uniseriate; macroconidia 4–5 μm long; perithecia pure black; thallus metallic bright green to dark green.....**6. *S. nitidula*** (Yunnan Province; Aptroot et al. 2003)

11'. Ascospores 10–18 μm long, biseriate; macroconidia 4–7 μm long; perithecia greyish black (covered by thin, thallus layer); thallus dark green to greenish brown

12. Thallus with distinct lobes leaving small to large interspaces, usually greenish brown; perithecia mostly wart-shaped...**10. *S. subtilissima*** (Hainan Province, Hongkong, Yunnan Province; Santesson 1952, Aptroot and Seaward 1999, Aptroot et al. 2003)

12'. Thallus with indistinct, completely confluent lobes leaving very small interspaces, usually dark green; perithecia mostly conical.....**11. *S. maculata*** (Yunnan Province; Aptroot et al. 2003)

9'. Thallus thicker (15–80 μm), pale greenish grey to bright green, not bordered by thin, black line

13. Ascospores small (8–15 × 2–3 μm); macroconidia 4–10 μm long....**7. *S. concreta*** (Yunnan Province; Aptroot 2003, Aptroot et al. 2003)

13'. Ascospores larger (12–25 × 4–7 μm); macroconidia 8–20 μm long

14. Perithecia 0.5–1.2 mm, prominent; ascospores usually uniseriate.....**12. *S. macrocarpa*** (Yunnan Province; Wei 1991, Aptroot et al. 2003)

14'. Perithecia 0.3–0.6 mm; ascospores usually biseriate
15. Macroconidiomata in groups
16. Thallus thin, 20–30 µm; asci 60–70 × 8–11 µm....**13. *S. antillarum*** (Guangxi Province, Hainan Province, Yunnan Province; Jiang et al. 2016)
16'. Thallus thick, 30–45 µm; asci 45–65 × 10–12.5 µm....**14. *S. guangxiensis*** (Guangxi Province; In this paper)
15'. Pycnidia solitary
17. Thallus pale greenish to bluish grey, 30–70 µm thick....**15. *S. subelegans*** (Yunnan Province; Wei 1991, Wei and Jiang 1991)
17'. Thallus bright green
18. Thallus 20–80 µm thick; asci 60–80 × 8–12 µm .....**16. *S. smaragdula*** (Fujian Province, Guizhou Province, Hubei Province, Hunan province, Yunnan Province; Santesson 1952, Wei 1991, Aptroot 2003, Aptroot et al. 2003)
18'. Thallus 10–25 µm thick; asci 50–65 × 8–12 µm; macroconidia with acute ends.....**17. *S. acuticonidiarum*** (Guangxi Province, Yunnan Province; In this paper)

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